

# Introduction: Utilization of Higher Plant Systems as Monitors of Environmental Mutagens

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Research over the past 10 years has clearly demonstrated the presence of mutagens among the numerous man-made and naturally occurring chemicals in our environment. These mutagens occur in all classes of chemicals, including foods, drugs, cosmetics, pesticides, household and industrial chemicals as well as in pollutants of both air and water. More recently, a high correlation has been found between carcinogenic and mutagenic activity; at least 90-95% of chemical carcinogens are mutagens. There is a widespread expectation that the discovery of mutagenic activity in chemical screening programs may alert us not only to mutagenic potential in man, but carcinogenic potential as well.

The types of genetic damage which can be produced are numerous and the specificity of chemical mutagens makes it possible for one type of effect to be produced predominantly or exclusively. Thus, any screening program must consist of a battery of tests capable of detecting nondisjunction, chromosome aberrations, gene mutations (point mutations as well as interstitial deletion), in addition to more subtle effects of DNA repair. In addition, since innocuous chemicals can be converted by mammalian metabolism to potent mutagens and carcinogens, these metabolites must be evaluated as well as the parent compounds.

Chemicals such as air pollutants present particular problems for mutagenicity testing using conventional microbial assays. Some of these problems can be overcome by using various higher plant systems. The general utility of these systems needs to be evaluated in terms of the types of genetic damage which can be detected, relative sensitivity, and general utility for use in mutagen screening and monitoring.

This workshop on higher plant systems as monitors of environmental mutagens was organized to provide a forum for generating new ideas and concepts for the utilization of higher plant systems to detect environmental mutagens. Such plant systems provide a unique resource which should be utilized more extensively as indicators of mutagenic activity. The workshop has been organized to review the major problem areas in environmental mutagenesis, to provide illustrative examples of areas where various plant systems have already been put to use, and to identify additional approaches for exploratory research.

Concern over the presence of mutagens in the environment became widespread about 10 years ago with the realization that there are genetically active chemicals in just about every chemical class known to man (1-3). These include food additives, drugs,

pesticides, cosmetics, air and water pollutants, as well as household and industrial chemicals. Concern was also enhanced by the discovery of supermutagens which can provide high frequencies of gene mutations at high levels of survival. In other words, high frequencies of mutations are produced at exposure levels which result in low or negligible levels of toxicity to the cell. There was great concern that some chemicals might be in widespread distribution because they had passed through traditional toxicological screening procedures or because they were never tested at all. Concern was amplified by the realization that because of the marked specificity of some supermutagens, man could be exposed to chemicals which could be potent inducers of gene mutations in the germ line without exhibiting other types of more visible genetic damage or cell killing effects that would alert us to their presence.

In addition, because of the high correlation that has been found between carcinogenic and

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mutagenic activity and the fact that many mutagens are also teratogens, widespread human exposure to undetected mutagens could be responsible not only for present frequencies of genetic disease in the human population but birth defects and cancer as well.

## Mutagenicity of AF-2

Probably the best illustration of the problem that we face is given by the chemical AF-2 (4), a nitrofur derivative used as a food preservative in Japan since 1965. AF-2 was used as a food preservative for soybean curd, fish, and meat sausage and many other foods considered staples in the Japanese diet. In 1973, Japanese scientists discovered that AF-2 was a potent mutagen in *E. coli*, as well as human cells in culture. Other experiments in this country rapidly confirmed the Japanese data in yeast and *Neurospora* and then in *Salmonella*.

The Japanese Ministry of Health was gravely concerned about the effect of this exposure, since it affected essentially every person in the Japanese population. In late August 1974, Japanese scientists also found that AF-2 produced cancer of the forestomach in mice (5). On the basis of all these data, the use of AF-2 as a food preservative was immediately banned; as a result of this ban, all foods containing AF-2 were removed from the market.

This finding, as well as the discovery in exploratory experiments of other potent mutagens in widespread distribution, showed that the concern expressed by geneticists in the mid-60's was valid, and attempts were made to organize and focus work in this area through the formation of Environmental Mutagen Societies in North America, Europe, Japan, India, the Soviet Union, and most recently in Australia.

In addition, last year a Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) was organized to do essentially the same job of protecting the human population from exposure to environmental chemicals that ICRP has done for radiation exposure for the past 25-30 years (6).

The primary emphasis in this new field of environmental mutagenesis has been to develop better assay systems to permit screening of large numbers of chemicals in the environment which have never been tested. Another goal has been to develop tests which can be performed directly on man to determine the effects of exposure on both somatic and germ cells in high-risk worker populations. Research in a third area has been directed towards determining whether there is a correlation between carcinogenic, mutagenic, and teratogenic activity.

Unlike radiation, which produces all types of genetic damage—nondisjunction, chromosome aberrations and gene mutations—chemicals can be highly specific and produce predominantly, if not exclusively, a particular class of genetic damage. Therefore, a battery of tests is required for a comprehensive evaluation of mutagenic potential rather than any single assay system. It is also important to test mammalian metabolites as well as the parent compounds, since chemicals can be activated in the mammalian liver and other organs to mutagenic derivatives. Techniques developed in the early 70's by using organ homogenates or microsomal fractions derived from various mammalian organs makes it possible to activate chemicals *in vitro*. By coupling these *in vitro* activation systems with various microbial assay systems it is now possible to test environmental chemicals as well as their mammalian metabolites for their ability to produce different types of genetic damage (2).

## Mutagenicity of Chemical Carcinogens

Using *Salmonella typhimurium* (as a tester set of histidine-requiring strains TA1535, TA1537, TA1538, TA98 and TA100) and these techniques for *in vitro* metabolic activation, research groups in the United States, England and Japan have shown in independent studies that some 90-95% of chemical carcinogens are also mutagens (7). We still do not know what percentage of mutagens are also carcinogens, but there is a general consensus among workers in the field that these short-term tests for mutagenic activity can be used not only to establish mutagenic potential in man but carcinogenic potential as well.

This potential for predicting adverse effects in the human population on the basis of short-term tests has lead to widespread interest in their use by regulatory agencies of our government to insure safety from exposure to chemicals in our environment. The recent development of mutagenicity testing guidelines for testing of pesticides for effective enforcement of The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) is a good example of how these tests procedures with laboratory organisms can be put to practical use.

Genetically active pesticides represent not only a hazard to the human population but to other organisms as well. Recent data on the herbicide Atrazine, for example, not only show it to be mutagenic to the plant that it was designed to "protect," but show extracts from corn plants grown on Atrazine-treated soil to be mutagenic in assays on laboratory organisms as well (8).

# Trends in Environmental Mutagenesis and Genetic Toxicology

The fields of environmental mutagenesis and genetic toxicology have developed very rapidly over the last 10 years and research can be divided into four main areas.

## Basic Mechanisms

Research in this area is designed to permit a better understanding of mechanisms by which mutations are produced. Particularly meaningful is research on the study of repair of different types of lesions in DNA as well as the effects of defective repair by utilizing repair-deficient organisms.

## Rapid Screens

Research in this area is directed towards development of assay systems which can provide a comprehensive evaluation of numerous untested chemicals in widespread distribution. How to test and what to test are the main problems in the development of screening programs that are (a) rapid and inexpensive, (b) can test the original chemical as well as its mammalian metabolites, and (c) which can detect all classes of genetic alteration including nondisjunction, chromosome rearrangements, gene mutations as well as effects on DNA repair and somatic recombination.

## Environmental Monitoring

Research in this area is directed towards the development of assay systems which can detect mutagens in air and water pollutants. This is particularly difficult, since the pollutants are often complex mixtures of chemicals which occur often in low concentrations. Detection of mutagenic activity in these cases requires very sensitive assay systems when ambient concentrations are evaluated. Concentration, elution, and even chromatographic separation of both air and water pollutants is being tried in many laboratories as alternative approaches.

## Risk Estimation

Research in this final area is designed to evaluate the risk of exposure to chemicals with identified potential in mass screening programs. Such assays usually require experiments in whole animals to determine whether the various types of genetic damage which can be induced will be transmitted to

progeny. Good examples of such tests are the mouse specific locus test for gene mutations and the mouse heritable translocation test for chromosome rearrangements.

## Utility of Plant Systems

Plant systems seem especially well suited for research in at least the areas of basic mechanisms, screening, and environmental monitoring. Lower plant systems are being used in screening programs to detect effects of DNA repair, point mutations, mitotic recombination, and gene conversion. Rapid tests have been devised which can be combined with *in vitro* techniques for *in vitro* metabolic activation using mammalian microsomes.

The most promising area for future development is a screening test for nondisjunction. Development of such an assay is absolutely essential for a comprehensive screen, since chemicals exist which can cause nondisjunction but not other classes of genetic alterations. No agents are known which cause chromosome breakage but not gene or point mutations; because of this, the lack of assay systems to detect chromosome breakage in this battery of tests is not considered as important.

Equally promising is the utilization of various plant systems for evaluation of air and water pollutants. In the paper on the *Tradescantia* Mobile Laboratory, we will hear how the *Tradescantia* stamen hair system is being used to evaluate the mutagenicity of various air pollutants (9). This assay system detects gene mutations in somatic cells and its sensitivity makes it especially suitable as a detector of weak mutagens and chronic levels of exposure.

In summary, the objective of organizing such a workshop and gathering this group of experts together is to discuss the important problem areas in this rapidly developing field of environmental mutagenesis with the hope that we can identify additional plant systems which can be used to provide unique or confirmatory genetic data. In addition, we hope not only to encourage better use of data on genetic effects of environmental chemicals already in the literature but also to identify systems with potential which are worthy of further exploratory research and financial support.

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